Journal of Genetics (2020)99:70
https://doi.org/10.1007/s12041-020-01226-6

RESEARCH ARTICLE

Population structure and diversity assessment of barley (*Hordeum vulgare* L.) introduction from ICARDA

PAWAN KUMAR[1]*, PRABHA BANJAREY[1], REKHA MALIK[2], A. N. TIKLE[1] and R. P. S. VERMA[2,3]

[1]*Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior 474 002, India*
[2]*ICAR-Indian Institute of Wheat and Barley Research, Karnal 132 001, India*
[3]*International Centre for Agricultural Research in the Dry Areas (ICARDA), 10000 Rabat, Morocco*
*For correspondence. E-mail: pawankumargpb1502@gmail.com.

Received 4 March 2020; revised 11 May 2020; accepted 31 May 2020

Abstract. This study was undertaken to measure the genetic diversity and population structure of 48 barley accessions introduced from ICARDA using 51 polymorphic simple sequence repeat (SSR) markers to select unique parents for breeding. The mean polymorphic information content was 0.491, suggesting high polymorphism for the selected SSR markers among the barley accessions. The population structure indicated a fine genetic base only with two major clusters. All accessions had 100% membership probability in their respective clusters. Analysis of molecular variance revealed that most (78%) of the variation was attributed between populations, while 22% was due to variation among individuals within populations. Neighbour-joining (NJ) tree was constructed using this distance matrix and two major clusters were observed in it. Cluster 1 had all hulled barley accessions and cluster 2 had all hulless barley accessions. Cluster 2 could be further divided into three subclusters. Principal coordinates analysis results were similar to the NJ tree, where the hulled and hulless barley accessions were grouped into separate clusters. This study established the existence of considerable genetic diversity among the 48 tested accessions. The selected genetic resources will be useful for barley breeding in India and other countries.

Keywords. simple sequence repeat; principal co-ordinates analysis; analysis of molecular variance; population structure; cluster; barley.

Introduction

Barley (*Hordeum vulgare* L.) is one of the first domesticated (about 10,000 years ago) cereals (Badr *et al.* 2000) most important crop of the world (FAOSTAT, http://faostat.fao.org) with an annual global production of about 145-m tons. Barley is an important feed, food and fodder crop as well as a major raw material for the brewing and malting industries. It is more tolerant to abiotic stresses (drought and soil salinity) and also better adapted to various environmental conditions than the other cereals, which make it a staple food in several dry and cold regions of the world (Baik and Ullrich 2008). Recent researches confirmed the beneficial effects of barley on human health and there results revitalized interest in preferred use of barley in human diets (Brockman *et al.* 2013; Sullivan *et al.* 2013). Global food demands are increasing day by day and to meet this in a period of climate change is one of the greatest challenges for agriculture. Barely is a cereal with a large genome and also a large number of varieties and accessions are available worldwide. Systemic evaluation at molecular level is a prerequisite to effectively use this available diversity in breeding as well as optimal conservation of the genetic diversity. Several studies have produced data for evaluation of genetic diversity using simple sequence repeats (SSRs) for a number of crops like wheat, barley, maize, sorghum and rice. The studies on barley (Russell *et al.* 2004; Baek *et al.* 2003; Maestri *et al.* 2002 and Amezrou *et al.* 2018) stipulated that the various diversity parameters have significant variations. Genetic variability is deteriorating day by day due to the continuous crossing with elite genotypes and this may lead to increasing vulnerability to the adverse climatic changes which also limit the possibilities of further improvement. There is a need for finding novel sources of

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s12041-020-01226-6) contains supplementary material, which is available to authorized users.

Published online: 20 August 2020

genetic variation which can be utilized in breeding programmes to further increase the chances of improvement in both quality and quantity along with improvement in their response to climate change.

World's largest barley germplasm collections, i.e. USDA-ARS National Small Grains Collection (NSGC), comprised of 33,176 barley accessions which includes breeding lines, cultivars, landraces and genetic stocks from more than 100 countries. The NSGC barley core collection is a subset having ~10% of the entire collection and is being assessed for various agronomic traits, resistance to diseases and pests. Most of the cultivars of domesticated barley are hulled

Table 1. Chromosomal location, allelic variation, major allele frequency and PIC of the polymorphic SSR loci in barley accessions.

	Marker	Chromosome	T_m (°C)	No. of alleles	Major allele frequency	PIC
1	ScSSR10477	1H	60	4	0.479	0.570
2	Bmag211	1H	58	6	0.313	0.713
3	ABG500a	1H	58	4	0.375	0.636
4	Bmagc13	1H	58	2	0.583	0.368
5	Bmag105	1H	55	2	0.521	0.375
6	ABC252	2H	58	5	0.750	0.404
7	ABG58	2H	58	2	0.542	0.373
8	Bmac576	2H	53	3	0.500	0.511
9	Bmag749	2H	55	4	0.833	0.279
10	Bmag829	2H	55	4	0.313	0.684
11	EBmac640	2H	55	4	0.625	0.523
12	HVM36	2H	55	5	0.458	0.645
13	HVM54	2H	55	5	0.292	0.731
14	ABG471	3H	56	3	0.646	0.462
15	ABG70	3H	56	3	0.583	0.475
16	Bmac129	3H	58	6	0.271	0.781
17	Bmag13	3H	58	3	0.375	0.582
18	Bmag225	3H	58	4	0.625	0.523
19	Bmag603	3H	55	2	0.667	0.346
20	Bmag877	3H	55	4	0.313	0.688
21	Ebmac541	3H	58	3	0.417	0.566
22	GMS27a	3H	60	2	0.625	0.359
23	EBmac705	3H	55	2	0.521	0.375
24	ABG500b	4H	55	2	0.667	0.346
25	HVM40	4H	55	5	0.354	0.692
26	HVM67	4H	55	4	0.333	0.611
27	MWG634	4H	58	2	0.729	0.317
28	Bmac175	4H	58	2	0.542	0.373
29	ABC302	5H	58	3	0.688	0.411
30	ABC483	5H	56	2	0.708	0.328
31	ABG712	5H	58	2	0.604	0.364
32	Bmag222	5H	58	3	0.375	0.590
33	Bmag223	5H	58	3	0.521	0.542
34	Bmag387	5H	58	2	0.542	0.373
35	Bmag760	5H	55	4	0.396	0.662
36	Bmag812	5H	55	5	0.417	0.669
37	GMS61	5H	60	2	0.729	0.317
38	Bmac163	5H	55	3	0.479	0.478
39	MWG684	6H	56	5	0.313	0.735
40	MWG798	6H	55	3	0.500	0.535
41	HVM14	6H	55	4	0.625	0.510
42	ABG387b	6H	60	2	0.667	0.346
43	Bmac40	6H	58	3	0.750	0.354
44	Mwg402	7H	55	3	0.417	0.582
45	Bmag273	7H	55	3	0.917	0.150
46	Bmac64	7H	58	4	0.521	0.520
47	Bmag110	7H	58	2	0.521	0.375
48	Bmac162	7H	58	5	0.375	0.702
49	Bmac167	7H	55	3	0.563	0.496
50	Bmac224	7H	55	2	0.667	0.346
51	ABG380	7H	55	2	0.583	0.368
	Mean			3.27	0.53	0.49

PIC, polymorphism information content.

(caryopses covered with hull), but some cultivars are free-threshing called hulless barley. Hulled barley is preferred for brewing and animal feed while the hulless barley is important for human food.

There are number of ways to assess the genetic diversity like morphological markers, biochemical or isozymes and DNA-based markers. A wide range of molecular markers are available for genotyping in various crops. SSR markers are feasible markers of choice for diversity assessment due to their easy use, abundance, codominance, cheaper and high polymorphism index (Powell *et al.* 1996). The current study was designed to determine the genetic relationship and population structure of 48 *Hordeum vulgare* introduced from ICARDA, using SSR markers to identify genetically divergent genotypes for utilization in breeding programme.

Materials and methods

The 48 barley accessions introduced from ICARDA, Lebanon, grown at experimental field of ICARDA-India Research Platform (IRP), Amlaha, (table 1 in electronic supplementary material at http://www.ac.in/jgenet/) were used. In randomized block design (RBD) with two replications in six row plots of 25 cm distance between rows. Sowing was done during the *rabi* (winter) season of 2014–2015. In case of fertilizer application, 50% of nitrogen (30 kg), full dose of phosphorus (60 kg) and potassium (40 kg) were applied as basal dose and with the remaining nitrogen top dressing of was done after 30 days of sowing. Crop was maintained under rainfed condition.

Genotyping studies

Molecular work was performed at biotechnology laboratory of ICAR-Indian Institute of Wheat and Barley Research, Karnal. Equal number of fresh young leaves (two-week-old seedlings) of at least six plants from each genotype was bulked for DNA extraction. Total genomic DNA was isolated using the modified CTAB method (Saghai-Maroof *et al.* 1984). The DNA samples were analysed both qualitatively and quantitatively using 0.8% agarose gel electrophoresis. A total of 150 SSR markers were selected from different locations of each linkage group of barley genome, of which only 51 markers showed polymorphism (table 1). The sequence of these markers, their annealing temperature (T_m), amplified fragment and PCR conditions were obtained from website (https://wheat.pw.usda.gov/cgi-bin/GG3/browse.cgi?class=marker). PCR reaction was conducted in a reaction volume of 10 μL containing 1x PCR buffer, 200 mM dNTPs, 0.25 μM of primer, 2 mM $MgCl_2$, 1 U *Taq* polymerase and 50 ng templates DNA. PCR amplification was performed using Biorad S1000 thermal cycler. PCR products were resolved by electrophoresis on 2.5% agarose gels (HiMedia, Mumbai, India) at 4 V/cm in 0.5x TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100-bp DNA ladder (New England Biolabs, Hitchin, UK) and corroborated with the reported amplified fragment size of respective molecular marker. The occurrence of 'null' alleles was verified by reamplification under similar PCR conditions. Gels were stained with ethidium bromide (0.5 μg/mL) and DNA banding patterns were visualized under UV light (Syngene Synoptics, Frederick MD, USA).

Scoring of SSR markers and assessment of genetic diversity

Genetic diversity was assessed using GenAlex v. 6.5 (Peakall and Smouse 2007). The following parameters were computed: total number of alleles per locus (N_a), number of effective alleles per locus (N_e), Shannon's information index (I), observed gene diversity (h), and unbiased gene diversity (uh) were determined using the protocol of Nei and Li (1979). Molecular weights for microsatellite products in base pairs were estimated and the summary statistics including the number of alleles per locus and frequency of major alleles were determined. Initial statistical analysis of the genotypic data was performed using the software PowerMarker 3.25 (Liu and Muse 2005). For each marker, number of alleles, gene diversity, and polymorphism information content (PIC) values were calculated. The PIC value revealed the information prospective of a marker. A marker with PIC value of '1' showed different molecular weight in every line, and a monomorphic marker had a '0' PIC value. Allele frequency and count were obtained using PowerMarker. Pairwise genetic distance between the accessions was calculated following Nei *et al.* (1983) method using the software PowerMarker 3.25. Distance matrix was used to construct the dendrogram using the neighbour-joining (NJ) option in PowerMarker. The dendrogram file obtained from PowerMarker was visualized and edited using MEGA7

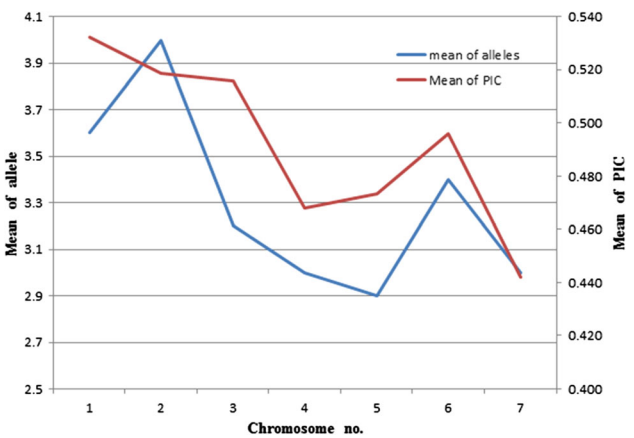

Figure 1. Genetic diversity (mean PIC and mean number of allele) among seven chromosomes of barley on the basis of microsatellite markers.

(Kumar *et al*. 2016). For further genetic analysis, the population-structuring pattern was conducted using allele molecular weight data in binary format (1, allele presence; 0, allele absence) and entered into a matrix. Principal co-ordinates analysis (PCoA) was calculated using PAST software (Hammer *et al*. 2001).

Analysis of population structure

The analysis on population structure of 48 barley (*H. vulgare* L.) accessions was also performed using the software Structure 2.3.4 (Pritchard *et al.* 2000). An admixture model was selected to calculate the number of subpopulations or *K* value. Initially, five runs for each value of *K* ranging from 1 to 20 were conducted with 10,000-length burn-ins and equal number of replications. Estimation of number of subpopulations (*K* value) was done by plotting the distribution of deltaK (Δ*K)*, which is an *ad hoc* statistic based on the rate of change in the log probability of data between successive *K* values (Evanno *et al.* 2005). The value of Δ*K* was calculated as mean of absolute values of difference between successive likelihood values of *K* divided by the standard deviation of *L* (*K*). The Δ*K* plots were made using the Structure Harvester program (Earl and Vonholdt 2012).

Result

Out of 150 SSR markers used to screen the present population of barley, only 51 were polymorphic with banding pattern of more than 1 band for each marker (table 1). These 51 markers generated 158 polymorphic loci as genotypic data with an average of 3.275 alleles per SSR locus and these were further used to assess the genetic diversity. Average major allele frequency was 0.532, with minimum and maximum values of 0.271 and 0.917, respectively. The PIC value for SSR markers ranged from 0.150 to 0.781, with an average PIC value of 0.491. The SSR markers used in the present study were distributed throughout the seven chromosomes of the barley. Average mean of alleles and PIC (figure 1) showed that the chromosome 2H has highest mean of alleles followed by 1H and 6H, while highest mean of PIC was recorded in 1H followed by 2H and 3H. However, 5H had least mean for alleles followed by 4H and least PIC was recorded in 7H with means of alleles too lower. Higher mean of alleles shows the diversity or variation present on the chromosome and higher PIC value shows that markers are more informative. Genetic diversity between barley accessions based on type of husk are provided in table 2. N_a (number of different alleles per locus) were 2.392 and 2.294 for hulled and hulless barley population, respectively, with a mean of 2.343. However, number of effective alleles per locus was 2.048 and 1.970 in hulled and hulless barley population, respectively with a overall mean of 2.009. While, N_P (number of private alleles per locus) were 0.647 for hulled barley population and 0.549 for hulless barley population with mean value of 0.125. Mean value of *I* was 0.716 with 0.746 and 0.685 for hulled and hulless population, respectively. N_P (frequency of private alleles) was higher for both hulled and hulless barley. Gene diversity (*h*) value was 0.483 for hulled barley population while 0.440 for hulless barley.

Table 2. Genetic diversity of 48 barley accessions classified by type of barley.

Type of barley	N_a	N_e	N_P	I	*h*	*Uh*
Hulled barley	2.392	2.048	0.647	0.746	0.483	0.504
Hulless barley	2.294	1.970	0.549	0.685	0.440	0.460
Mean	2.343	2.009	0.125	0.716	0.462	0.482
SE	0.068	0.058	0.117	0.027	0.015	0.016

N_a, number of different alleles per locus; N_e, number of effective alleles per locus; N_P, number of private alleles per locus; *I*, Shannon's information index; *h*, gene diversity; *uh*, unbiased diversity.

Population structure analysis

Structure 2.3.4 was used for the analysis of population structure in the barley diversity for a panel of 48 accessions. It was not feasible to identify the number of clusters using the Ln plot for *K* (figure 2a), so a plot for D*K* was used to identify the number of subpopulations (figure 2b). Based on the admixture model, 48 (*H. vulgare*) barley accessions were placed in clusters using 80% membership probability as a threshold value. Two clusters were identified for all the accessions of barley (figure 3). Each group has clustered 24 accessions of the germplasm. All the accessions of a single cluster have shown 100% membership probability in respective cluster. These groups were formed irrespective of the type of barley, i.e. hulled and hulless. Both the clusters had equal number of hulled and hulless accession of barley. This is because both type of barley consist of same genome except *nud* gene in hulless barley on 7HL chromosome, which is responsible for loose husk of hulless barley. SSR markers used for screening were not close enough or linked to *nud* gene and thus this gene did not make difference in population of the germplasm.

Phylogenetic and PCoA

Genetic distance was obtained from the NJ analysis of genotypic data using PowerMarker software. The genetic distance in the complete panel ranged from 0.1941 to 0.9983 with an average of 0.7333 (table 2 in electronic supplementary material), suggesting that the panel consists of very diverse accessions. A NJ tree was constructed using this distance matrix (figure 4). Two major clusters were observed

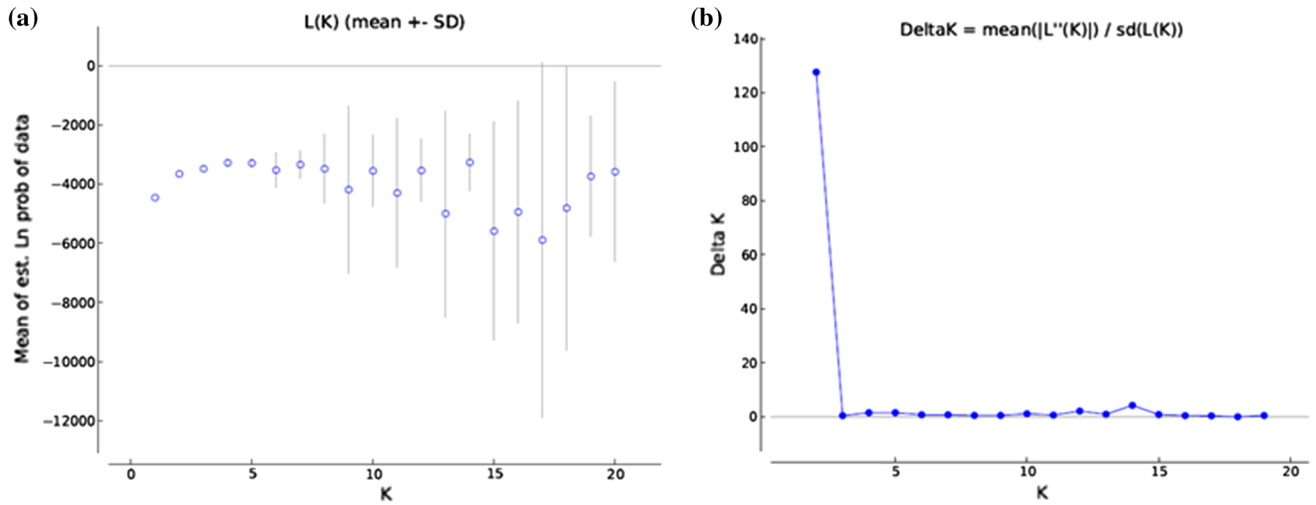

Figure 2. (a) Probability of data (Ln) for number of clusters (K) ranging 1 to 20 (b) Estimation of number of clusters using delta K values for K ranging 2 to 20 by method proposed by Evanno *et al.* (2005).

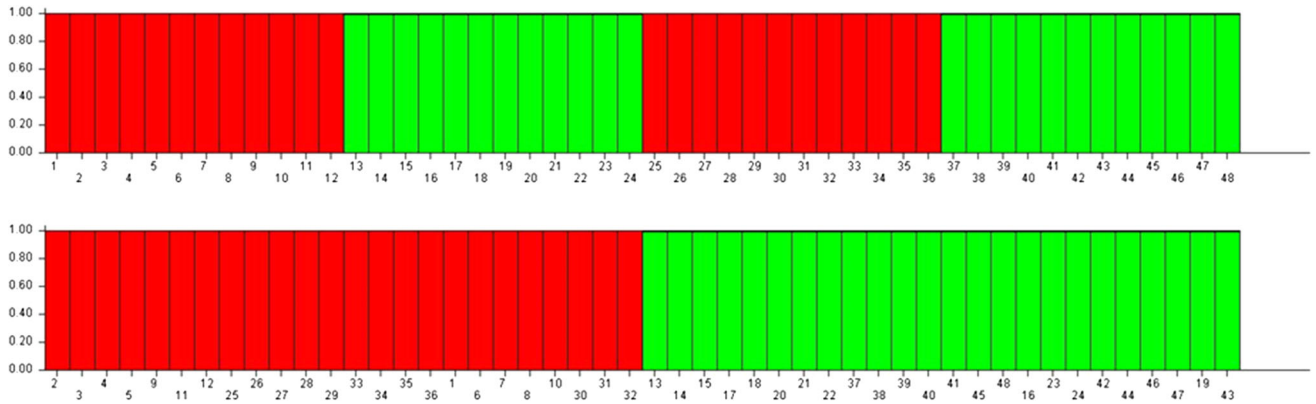

Figure 3. Q plot showing clustering of 48 barley accessions into two clusters based on co-dominant genotypic data (SSR) using STRUCTURE analysis. A vertical bar represents each accession. The coloured sections in a bar indicate membership coefficients of the accession in different clusters.

in the NJ tree. Both the cluster had 24 genotypes each. Hulled barley genotypes were clustered together in a single cluster and hulless barley into the other. The cluster of hulless barley was further divided into three subclusters, leading to a total of four clusters.

To further evaluate the genetic relationships between the barley accessions using PAST software, the PCoA analysis was carried out. It was observed that 20% of variation was accounted for first two axes of PCoA, and the accessions were grouped into four major clusters on first two axes of PCoA. Results similar to the NJ tree were obtained where hulled and hulless barley accessions were grouped into separate clusters and hulless barley cluster was further divided into three subclusters.

The phylogenetic tree constructed using NJ analysis on 48 (*H. vulgare*) accessions separated the accessions into two major clusters. To compare STRUCTURE sub-populations with NJ analysis based tree, red and green symbols were given with name of accessions to present SRUCTURE sub-populations (figure 4). There was partial agreement between the two estimates. Groups 1 and 2 from Structure were spread across both the clusters of dendrogram. The membership probability obtained from Structure analysis suggested that both groups were genetically distinct from each other. A PCoA plot was constructed using the diversity panel, and accessions were circled to show the Structure groups (figure 5). The results of PCoA were in complete agreement with results inferred from NJ tree (figure 4), group 1 was separated from other accessions and formed tight and clear cluster. All other accessions of group 2 were loosely clustered and can be further divided into subclusters as in dendrogram. The first two axes of PCoA explained 20% of the total genetic variation between *H. vulgare* accessions.

Analysis of molecular variance (AMOVA) revealed that differences between groups obtained from Structure analysis were highly significant, with 78% of the total variation contributed by between-group variance. However, 22%

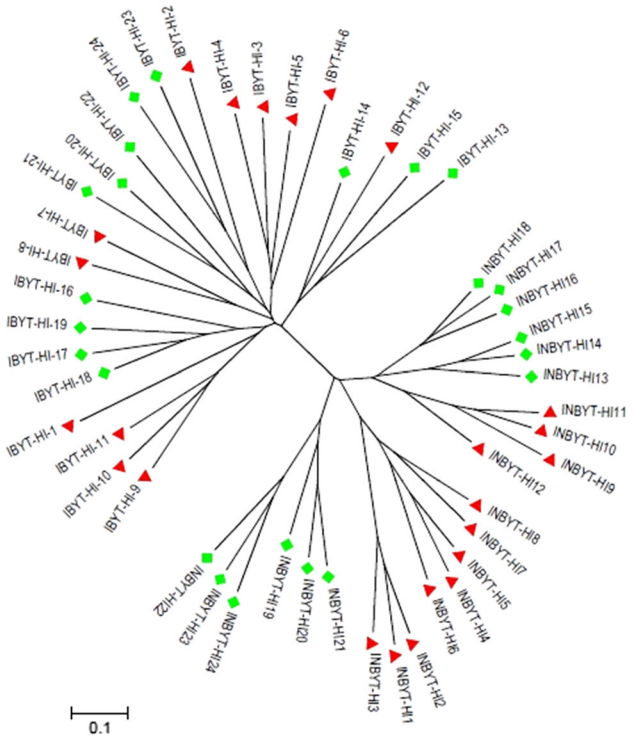

Figure 4. Phylogenetic tree obtained from neighbor-joining (NJ) analysis on 48 barley accessions. Accessions are pointed with coloured symbols based on groups identified from STRUCTURE analysis.

variation was attributed to diversity between individuals within a group (table 3). The F_{ST} value for the whole population (0.778) was highly significant at $P < 0.0001$. Pairwise F_{ST} values revealed that accessions in group 1 were genetically significantly, far from another group.

Discussion

The genetic upgrading of yield and contributing traits in crop are influenced by the genetic diversity available within the crop species and the barley accession with high levels of genetic variation found in this study are valuable resources for expansion of the genetic base and for attaining prompt gains during barley breeding in India. Genetic diversity in the 48 accessions of *H. vulgare* L. introduced from ICARDA, Lebanon was evaluated using 75 SSR primer pairs distributed across the genome, of which only 51 SSR primers pairs showed polymorphism (two or more loci) that were used in the final analysis. A total of 2446 alleles were observed across 158 loci among 48 accessions, with an average of 3.27 alleles per SSR locus. This confirm the presence of favourable allelic diversity, which is considered necessary for assessment of genetic diversity. The mean number of alleles (3.275) obtained in the study were significantly similar to the mean number of alleles per locus (2.5 to 3.5) reported by Bengtsson *et al.* (2017) in his study

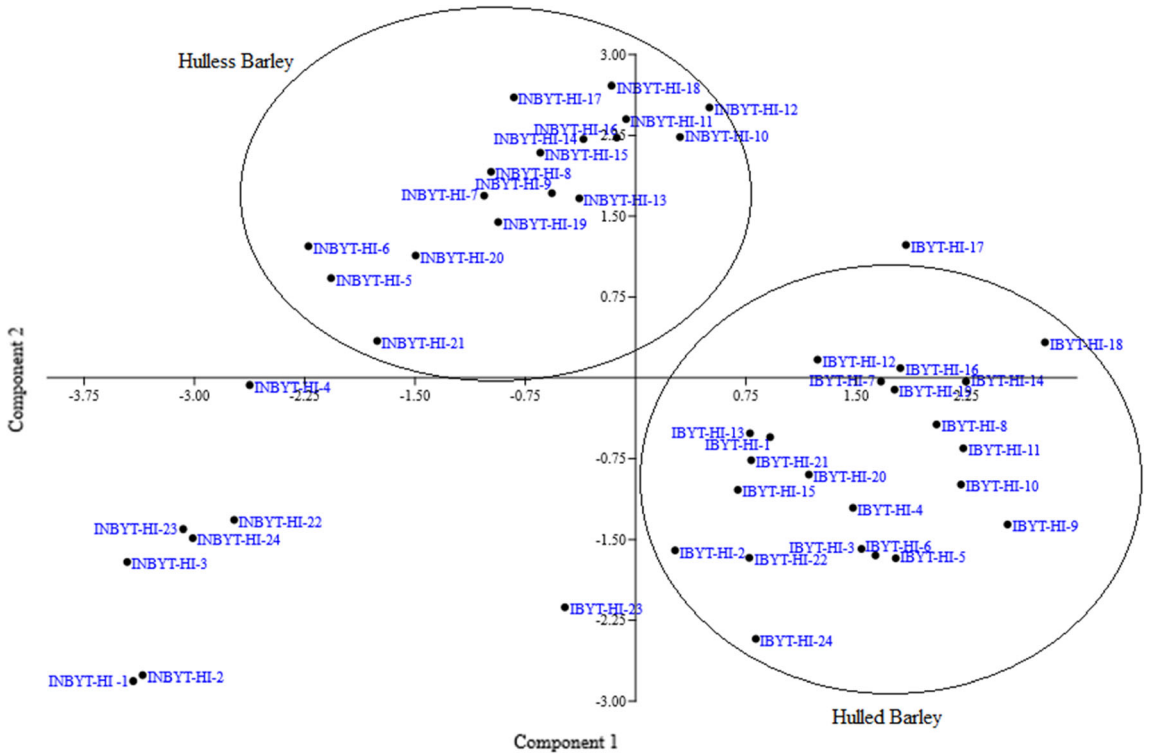

Figure 5. Two-dimensional PCoA of 48 barley accessions.

Table 3. Analysis of molecular variance among and within the 48 barley accessions.

Source	Degree of freedom	Sum of square	Mean sum of square	Estimated variance	Percent of variance
Among populations	1	574585.375	574585.375*	23660.116	78
Within populations	46	310158.708	6742.581*	6742.581	22
Total	47	884744.083		30402.697	100

*Significant at the 0.0001 probability level.

on Nordic spring barley. While much higher mean number of alleles (5.5) per locus was reported by Ivandic *et al.* (2002) from wild barley and Maniruzzaman *et al.* (2014) found 7.8 mean number alleles per microsatellite/genotypes locus. Since, in our study, all accessions are from same centre of origin and their pedigree gene pool is also small, we got fewer alleles per locus as compared to earlier studies in which genotypes of various centre of origin and distinct parents were taken. The PIC value of the SSR markers ranged from 0.150 to 0.781, with an average of 0.491. High PIC value showed that the SSR markers used in this study were informative for diversity of assessment of barley population. Bengtsson *et al.* (2017) reported average PIC value of 0.46 for SSR markers in his study. Malysheva-Otto *et al.* (2006) reported varying PIC values in different population in their study with an overall average of 0.69 of all four populations. Hua *et al.* (2015) reported PIC ranging from 0.064 to 0.815 with an average of 0.549 in diversity of coloured barley. N_a (number of different alleles) was 2.392 and 2.294 for hulled and hulless barley population, respectively, with a mean of 2.343. Number of effective allelers per locus was 2.048 and 1.970 in hulled and hulless barley population, respectively with a overall mean of 2.009. Manjunatha *et al.* (2011) reported similar results for N_a and N_e in his study in barley population from three different state of India. Bellucci *et al.* (2013) found 3.75 N_a and 2.03 N_e in one of the barley population and 3.42 N_a and 2.23 N_e for another population of barley in his study. Mean value of I was 0.716 with 0.746 and 0685 for hulled and hulless population, respectively. N_P (number of private alleles per locus) was 0.647 for hulled barley population and 0.549 for hulless barley population with mean value of 0.125. The presence of high number of private alleles supports the broad genetic base of the present germplasm. Private alleles used for estimating the gene flow between the populations (Barton and Slatkin 1986), and their estimation have applications in conservation genetics (Kalinowski 2004). Gene diversity (h) value was 0.483 for hulled barley population while 0.440 for hulless barley. Similar range of I and h was recorded by Manjunatha *et al.* (2011) in barley landraces and Hua *et al.* (2015) during diversity study of coloured barley. The genetic diversity study showed significant level of variation in the barley population of the study which can be used as parent material for the development and improvement of barley crop varieties.

Based on the admixture model, 48 *H. vulgare* accessions were placed into two clusters using 80% membership probability as a threshold value. All the accessions of a single cluster have shown 100% membership probability. Hua *et al.* (2015) also reported similar results in his study that most of the barley accessions were showing 100% membership probability to their respective clusters. The results of Structure were almost similar to NJ tree and as by all means PCoA accessions were clustered into two major clusters. Clusters formed by Structure grouped the accessions irrespective of type of barley, i.e. hulled and hulless. Both the clusters had equal number of hulled and hulless accession of barley. This is because both types of accessions are collected from the same origin. Barley consists of same genome except *nud* gene in hulless barley on 7HL chromosome which is responsible for loose husk of hulless barley. Therefore, at genomic level hulled and hulless barley accessions can be linked or closely associated with each other. Another reason for this can be that the SSR markers used for screening in the present study were not close enough or linked to *nud* gene and could not differentiate accessions in population. The size of germplasm was sufficient to conduct the Structure analysis as number of polymorphic markers have covered each chromosome of the genome. Manjunatha *et al.* (2011) also found similar results while assessing the population structure of 15 barley genotype using 15 SSR markers, and Jena *et al.* (2012) assessed the population structure of 51 cotton genotypes using 16 amplified fragment length polymorphism (AFLP) markers.

In the complete panel, the genetic distance ranged from 0.1941 to 0.9983 with an average of 0.7333, suggesting that the panel consists of very diverse accessions. A NJ tree was constructed using this distance matrix. Two major clusters were observed in the NJ tree, with both the clusters having 24 genotypes each. Hulled barley genotypes were clustered together in single cluster and hulless barley into the other. The cluster of hulless barley was further divided into three subclusters, leading to a total of four clusters. Similar results were found by Kim *et al.* (2005) in genetic diversity measurement of barley and grouped hulled and hulless barley into different cluster by random amplification of polymorphic DNA (RAPD) markers and Drikvand *et al.* (2012) clustered hulled and hulless barley separately irrespective of two row or six row barley in his study using molecular markers. PCoA was carried out to further evaluate the genetic relationships between the barley accessions. It was observed that 20% of variation was accounted for first two axes of PCoA, and the accessions were grouped into four major clusters on first two axes of PCoA. For confirmation

of cluster analysis, Malysheva-Otto *et al.* (2006) also used PCoA and found similar results with first two axes accounting for 8.9% and 5.9% of the total variance. While, in coloured barley diversity study, PCoA results indicated that the hulled and naked accessions within differently coloured barley populations could not be readily distinguished, possibly owing to the selection of individual materials and the difference in the number of individuals with hulled or naked kernels (Hua *et al.* 2015). Results similar to the NJ tree were obtained where hulled and hulless barley accessions were grouped into separate clusters and hulless barley cluster was further divided into three subclusters. Both in NJ tree and PCoA, hulled barley accessions were clustered tightly and hulless barley accessions were so loose that it can be further divided into sub groups. However, results of analysis of molecular variance also showed that in the present study variation among the population is much higher as compare to within population depicting that these accessions cannot be clustered further. Bengtsson *et al.* (2017) also recorded similar results in his study of Nordic spring barley for PCoA, cluster analysis and AMOVA. Although, less variation within population in present study is contradictory to results of Hua *et al.* (2015) showing that most of variation was among accessions within population that was because colour of barley did not contribute to variation much.

In conclusion, the present study reported the presence of rational variability among barley introductions, which could be exploited for future crop improvement. The results revealed that 51 SSR markers were highly polymorphic and distributed though out all the seven chromosomes of barley genome and significantly distinguished the tested barley accessions. The Structure, phylogenic cluster analysis and PCoA classified 48 barley accessions into two major distinct genetic groups irrespective of the husk type. The hulless barley accession were clustered loose in phylogenic tree, and PCoA revealing the presence of diversity among them and further divided into subclusters. The presence of significant number of private alleles suggested that these accessions can be utilized as sources of novel genes in barley breeding programmes. Hence, the information generated form the present study will contribute significantly to barley improvement programmes in India and other South Asian countries.

Acknowledgement

The authors gratefully acknowledge Mr Deepender Kumar (Ph.D. Scholar, ICAR-IIWBR, Karnal) for his assistance in statistical analysis.

References

Amezrou R., Gyawali S., Belqadi L., Chao S., Arbaoui M., Mamidi S. *et al.* 2018 Molecular and phenotypic diversity of a worldwide ICARDA spring barley collection. *Genet. Resour. Crop Evolut.* **65**, 255–269.

Badr A., Rabey H. E., Effgen S., Ibrahim H. H., Pozzi C., Rohde W. *et al.* 2000 On the origin and domestication history of barley (*Hordeum vulgare* L). *Mol. Biol. Evol.* **17**, 499–510.

Baek H., Beharav A. and Nevo E. 2003 Ecological-genomic diversity of microsatellites in wild barley (*Hordeum spontaneum*) populations in Jordan. *Theor. Appl. Genet.* **106**, 397–410.

Baik B. K. and Ullrich S. E. 2008 Barley for food: characteristics, improvement, and renewed interest. *J. Cereal Sci.* **48**, 233–242.

Barton N. H. and Slatkin M. 1986 A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* **56**, 409.

Bellucci E., Bitocchi E., Rau D., Nanni L., Ferradini N., Giardini A. *et al.* 2013 Population structure of barley landrace populations and gene-flow with modern varieties. *PLoS One* **8**, e83891.

Bengtsson T., Manninen O., Jahoor A. and Orabi J. 2017 Genetic diversity, population structure and linkage disequilibrium in Nordic spring barley (*Hordeum vulgare* L. subsp. vulgare). *Genet. Resour. Crop Evol.* **64**, 2021–2033.

Brockman D. A., Chen X. and Gallaher D. D. 2013 Consumption of a high β-glucan barley flour improves glucose control and fatty liver and increases muscle acylcarnitines in the Zucker diabetic fatty rat. *Eur. J. Nutr.* **52**, 1743–1753.

Drikvand R., Salahvarzi E., Salahvarzi A. and Hossinpour T. 2012 Study of genetic diversity among rainfed barley genotypes using ISJ markers and morphological traits. *J. Agric. Sci.* **4**, 137.

Earl Dent A. and Vonholdt Bridgett M. 2012 STRUCTURE HARVESTER: a website and program for visualizing structure output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**, 359–361.

Evanno G., Regnaut S. and Goudet J. 2005 Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620.

Hammer O., Harper D. A. and Ryan P. D. 2001 PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **4**, 9.

Hua W., Zhang X., Zhu J., Shang Y., Wang J., Jia Q. *et al.* 2015 A study of genetic diversity of colored barley (*Hordeum vulgare* L.) using SSR markers. *Genet. Resour. Crop Evol.* **62**, 395–406.

Ivandic V., Hackett C. A., Nevo E., Keith R., Thomas W. T. and Forster B. P. 2002 Analysis of simple sequence repeats (SSRs) in wild barley from the fertile crescent: associations with ecology, geography and flowering time. *Plant Mol. Biol.* **48**, 511–527.

Jena S. N., Srivastava A., Singh U. M., Roy S., Banerjee N., Rai K. M. *et al.* 2012 Analysis of genetic diversity, population structure and linkage disequilibrium in elite cotton (*Gossypium* L.) germplasm in India. *Crop Pasture Sci.* **62**, 859–875.

Kalinowski S. T. 2004 Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conserv. Genet.* **5**, 539–543.

Kim H. S., Park K. G., Baek S. B., Kim J. G. and Nam J. H. 2005 Genetic diversity measured by RAPDs in Korean barley germplasm pools. *Korean J. Crop Sci.* **50**, 131–141.

Kumar S., Stecher G. and Tamura K. 2016 MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**, 1870–1874.

Liu K. and Muse S. V. 2005 PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**, 2128–2129.

Maestri E., Malcevschi A., Massari A. and Marmiroli N. 2002 Genomic analysis of cultivated barley (*Hordeum vulgare*) using sequence-tagged molecular markers. Estimates of divergence based on RFLP and PCR markers derived from stress-responsive genes, and simple-sequence repeats (SSRs). *Mol. Genet. Genomics* **267**, 186–201.

Malysheva-Otto L. V., Ganal M. W. and Röder M. S. 2006 Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.). *BMC Genet*. **7**, 6.

Maniruzzaman M., Talukder Z. A., Rohman S., Begum F. and Amiruzzaman M. 2014 Polymorphism study in barley (*Hordeum vulgare*) genotypes using microsatellite (SSR) markers, Bangladesh. *J. Agric. Res*. **39**, 33–45.

Manjunatha T., Bisht I. S. and Bhat K. V. 2011 Genetic structure of hull-less barley (*Hordeum vulgare* L. subsp. vulgare) landrace populations from North-western Indian Himalayas. *Indian J. Biotechnol*. **10**, 25–32.

Nei M. and Li W. H. 1979 Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci*. *USA* **76**, 5269–5273.

Nei M., Tajima F. and Tateno Y. 1983 Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol*. **19**, 153–170.

Peakall R. and Smouse P. E. 2007 GENALEX6: genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol*. **6**, 288–295.

Powell W., Machray G. C. and Provan J. 1996 Polymorphism revealed by simple sequence repeats. *Trends Plant Sci*. **1**, 215–222.

Pritchard J. K., Stephens M. and Donnelly M. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Russell J., Booth A., Fuller J., Harrower B., Hedley P., Machray G. and Powell W. 2004 A comparison of sequence-based polymorphism and haplotype content in transcribed and anonymous regions of the barley genome. *Genome* **47**, 389–398.

Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W. L. 1984 Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci*. *USA* **24**, 8014–8018.

Sullivan P., Arendt E. and Gallagher E. 2013 The increasing use of barley and barley by-products in the production of healthier baked goods. *Trends Food Sci. Technol*. **29**, 124–134.

Corresponding editor: Manoj Prasad